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Stoichiometric Correlation of Malate Accumulation with Auxin-dependent K^+-H^+ Exchange and Growth in *Avena* Coleoptile Segments[1, 2]

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ABSTRACT

The action of auxin in the promotion of growth has been suggested in the literature to depend on cell wall acidification. In a former investigation by the present authors the electrochemical balance in auxin-induced proton extrusion was shown to be maintained by potassium net uptake. The present paper reports data demonstrating that the elongation of *Avena* coleoptile segments is accompanied by an accumulation of malate, which is stoichiometrically correlated with potassium uptake. We concluded that this malate accumulation is required in a mechanism regulating intracellular pH.

Auxin (IAA)-induced elongation of shoot cells is accompanied by a rapid release of protons from the cells (5, 21). External media of low pH (3–5) can mimic the action of auxin (3, 9, 25). These results have led to the suggestion that hydrogen ions play a role as second messengers in auxin action (9): acidification of the cell wall enhances its extensibility either by activating cell wall-loosening enzymes or by breaking some acid-labile links.

Indoleacetic acid is also known to stimulate the uptake of K^+ and Rb^+ ions into different tissues (14–16). In a previous paper (10) we demonstrated that IAA-promoted proton efflux is electrochemically balanced by a stoichiometric influx of K^+ ions. The presence of alkali, and, in particular, of K^+ ions synergistically stimulates IAA-induced growth (11).

A proton efflux of the observed magnitude must be associated with a mechanism regulating cytoplasmic pH. In many tissues a proton-cation exchange (or antiport), *e.g.*, during excess cation uptake, is associated with synthesis and subsequent accumulation of malic acid by dark CO_2-fixation via P-enole-pyruvate carboxylases (12). A similar mechanism is reported to be involved in K^+-H^+-exchange regulating stomatal guard cell movement (1, 18). On the basis of these and other results, Davies (6) and Raven and Smith (19) proposed a common mechanism for stabilization of intracellular pH, which is based on synthesis and breakdown, respectively, of organic acids, especially of malate. In this respect it may appear to be pertinent that IAA-promoted growth is accompanied by consumption of CO_2 (25) and that IAA enhances the fixation of $H^{14}CO_3^-$ by *Avena* coleoptile cells (4).

The present paper demonstrates that IAA-induced elongation growth is paralleled by a stoichiometric potassium and malate accumulation.

MATERIAL AND METHODS

Plant Material. Seeds of *Avena sativa* (cv. "Flemings Krone") were dehusked and soaked for 3 hr in three changes of aerated 0.5 mM $CaSO_4$. Subsequently, the seeds were placed on a steel screen floated on aerated 0.5 mM $CaSO_4$. Germination, preparation of tissues, and experiments were carried out under dim green light at 28 ± 1 C, except for a 3-hr exposure to red light at the beginning of the germination period.

Experiments. Using a double blade cutter 10-mm coleoptile segments were obtained from 96-hr-old seedlings (30–40 mm in length). The top 3 mm of the coleoptile were discarded and the next 10 mm were used in the experiments. The segments were preincubated in 0.5 mM $CaSO_4$ for 2 hr, randomized, and transferred to the test solutions, usually with 30 segments in 100 ml of solution. The solutions contained 1 mM tris + 1 mM KCl with or without 10 μM IAA; pH 7 was established by addition of some drops of 0.1 N H_2SO_4. At the end of the incubation time, the tissue was blotted dry and transferred to 100 ml of ice-cold bidistilled H_2O for 15 min to remove adhering ions, and then frozen.

Measurements. For growth measurements coleoptile segments were photographed in transmitted light before freezing, and their lengths were determined on the negatives projected onto a screen. The frozen samples were dried at room temperature under reduced pressure. Ion contents were determined from aqueous extracts; K^+ by flame photometry and Cl^- by electrochemical titration. Malate was estimated by recording the reduction of NAD^+ in the presence of malate dehydrogenase (photometrically) at 340 nm, as described by Hohorst (13). Initial amounts of ion and malate contents were obtained by analyzing coleoptile sections which were frozen after the 2-hr period of preincubation.

Statistical Treatment. All experiments were repeated three times or more with three parallels each. The significance of replicates within each individual experiment was assessed by Duncan's multiple range test at the 0.05 level. If the standard errors of the means (S.E.M.) exceeded the size of the symbols in the figures they were indicated as vertical bars. The regression line in Figure 3 was calculated using the method of least squares (r = correlation coefficient).

Due to the great variability of the material between different batches of tissue used for the individual experiments, a statistical

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[2] The result of this paper has been communicated orally at the 1974 conference of the Deutsche Botanische Gesellschaft, Würzburg, September 26, 1974.

treatment comprising results of separate individual experiments proved to be of no value. Results were considered significant, when repetitive individual experiments gave similar patterns of response, although absolute data (*e.g.*, slopes, maxima) were varying (see two individual experiments depicted in Fig. 1 or Fig. 2).

RESULTS AND DISCUSSION

The time courses depicted in Figure 1 show the effect of IAA on malate accumulation of the *Avena* coleoptile tissue. After 6 to 8 hr, a clear increase of malate levels in the tissue is observed in the presence of IAA. At shorter periods significant differences in malate levels were not obtained. In the experiment depicted by closed circles in Figure 1 malate accumulation extrapolates almost linearly to zero time. In the other experiment (open circles) there is an apparent lag. Buffering capacity of the cytoplasm may maintain the intracellular pH initially for some time without detectable accumulation of malate in spite of the very rapidly measurable proton efflux (5, 21). $^{14}CO_2$ dark fixation experiments are under way to collect more reliable data describing the events during the first 4 hr. At longer time periods malate accumulation continues during the entire experiment extending over 16 hr. In the absence of IAA there is a much smaller, but still significant, increase in malate levels of the tissue during the course of the experiment. This may be due to excess cation over anion uptake. In our experiments net K^+ uptake by the coleoptile tissue always considerably exceeded net Cl^- uptake (not shown) irrespective of IAA treatment. This is in contrast to data reported by Rubinstein and Light (22) and Rubinstein (23).

Malate accumulation is clearly correlated with growth of the coleoptile cylinders (Fig. 2). In some experiments (closed symbols in Fig. 2) growth ceased after 14 hr, whereas malate syn-

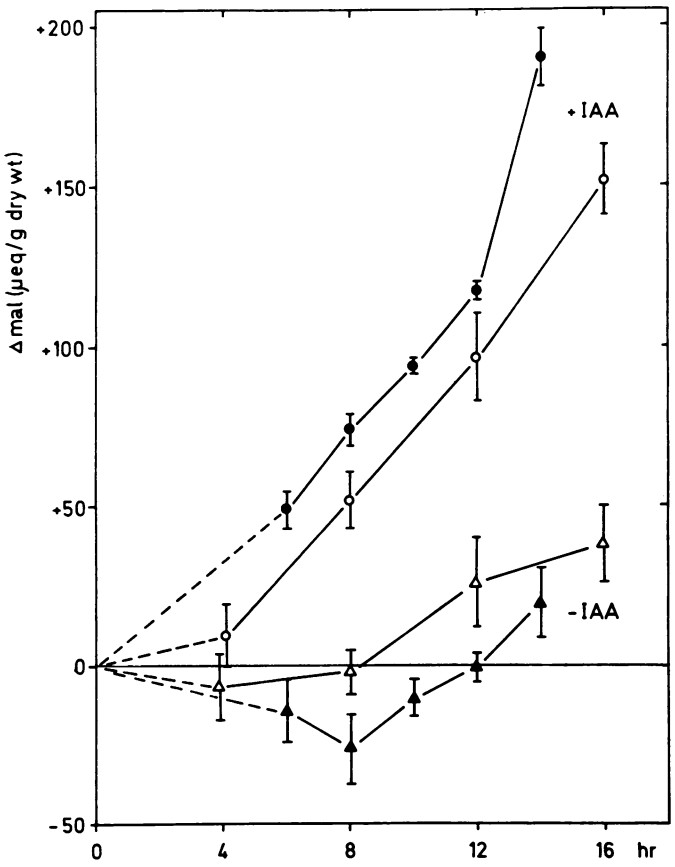

FIG. 1. Time course showing IAA effect on malate accumulation (Δmal) in *Avena* coleoptile segments (two individual experiments). The initial amounts of malate in the tissue were 162 ± 4 μeq/g dry weight in the experiment indicated by the open symbols and 210 ± 4 μeq/g dry weight in the experiment indicated by closed symbols.

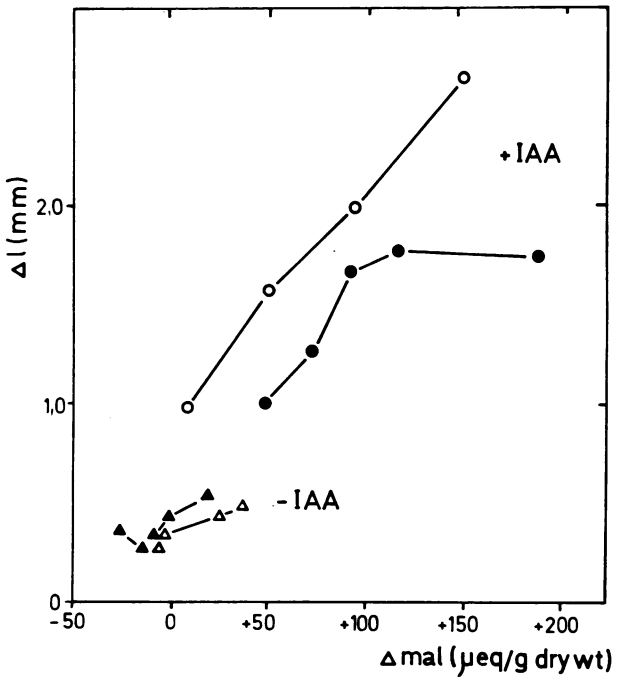

FIG. 2. Correlation between elongation growth (Δl) and malate accumulation (Δmal). Symbols are as in Figure 1.

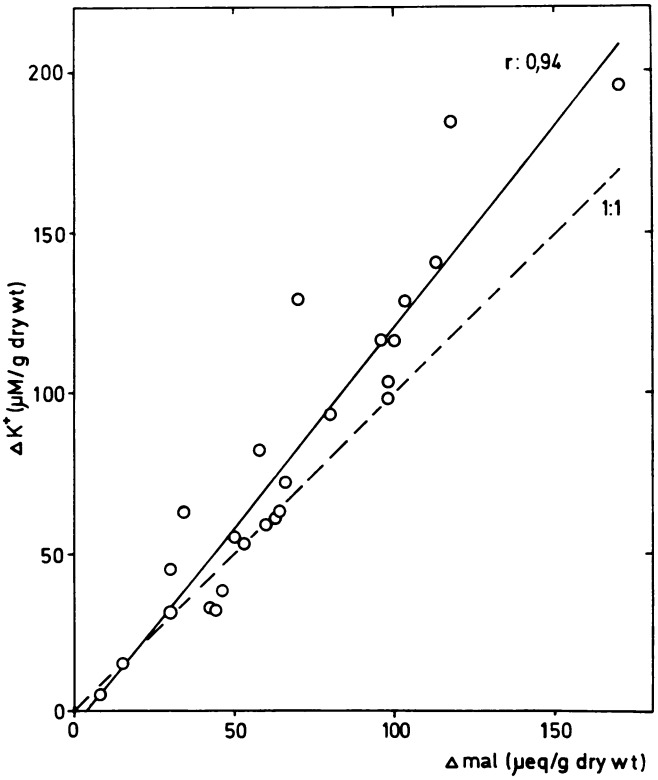

FIG. 3. Correlation of IAA-induced changes of K^+ and malate levels in *Avena* coleoptile segments. r = correlation coefficient.

thesis and K^+ uptake proceeded. Though malate accumulation in the absence of IAA is very small, it is accompanied by a small amount of elongation. Probably this "endogenous growth," which does not occur in the absence of KCl in the external solution (11), reflects a proton efflux and acidification of the cell wall that balances the excess K^+ over Cl^- uptake.

Figure 3 shows the stoichiometry between K^+ and malate accumulation. All data obtained of IAA-enhanced net K^+ uptake and malate accumulation are used in this figure to assess this correlation. The points fit the regression line quite well ($r = 0.94$). If net K^+ uptake were balanced stoichiometrically by malic acid synthesis with extrusion of the protons and accumulation of the malate^{2-} anions, theoretically a 1:1 relationship of μmole K^+ per μeq mal^{2-} or $\frac{1}{2}$ μmole mal^{2-} should be expected. The regression line shown in Figure 3 is very close to this theoretical expectation.

Malate synthesis and accumulation requires dark fixation of CO_2. It has been observed that the enhancement in growth is followed by an increase in CO_2 consumption (25). It is also well known that CO_2 promotes elongation in coleoptile segments (7, 17). The involvement of malate accumulation in auxin-induced elongation may account for the long term growth stimulations in response to CO_2. Rapid short term effects of CO_2 may arise from its acidifying effect (see also 2, 20).

We believe that the H^+-K^+-antiport, malate synthesis, and accumulation of K-malate are closely related events in IAA-stimulated growth and are probably regulated by feedback mechanisms. This leads to the speculation about where IAA might exert its primary action in this system, *i.e.*, at the level of one of the membrane transport mechanisms (H^+-K^+-antiport at the plasmalemma? K-malate transport at the tonoplast?), or at the level of the enzymes involved in malate synthesis. The idea of a primary action of hormones at the membranes has recently attracted much support (ref. 8, and citations therein). An effect on the mechanism of malate synthesis cannot be excluded before the appropriate tests with enzymes isolated from *Avena* coleoptiles have been performed.

CONCLUSIONS

In combination with former results (10) the present findings suggest that IAA activates a K^+-H^+-exchange pump, which is correlated with a stoichiometric synthesis and accumulation of malate to maintain cytoplasmic pH and cell turgor.

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